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Chemical constituents from *Lotus pusillus* Medik

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1. Subject and source

Family Fabaceae (Leguminosae) is one of the largest families of the flowering plants, with approximately 720–750 genera divided into 36–40 tribes and 18,000 species (Wink and Mohamed, 2003; Mabberley, 2007). The members of this family are distributed throughout the world and split into three subfamilies (Papilionoideae or Faboideae, Caesalpinioideae, and Mimosoideae) (Quezel and Santa, 1963).

The genus *Lotus* belonging to the subfamily Papilionoideae and the tribe *Loteae* comprises 14 sections with 120–130 species, mainly distributed around the Mediterranean region. It is well represented in Algeria by fifteen species, including *Lotus pusillus* Medik. (Quezel and Santa, 1963). *L. pusillus* Medik. also known as *Lotus halophilus* Boiss. & Spruner belonging to the section *Loteae* (Degtjareva et al., 2006), is a small plant that grows in sandy arid and desert pastures of Algeria (Quezel and Santa, 1963). The plant materiel was collected in April 2009 in the vicinity of Biskra (Algeria) and was identified by Pr Bachir Oudjehih, Agronomic Department of the University of Batna, where a voucher specimen has been deposited (N° 602/LCCE).

2. Previous work

Previous investigations on the chemical composition of *Lotus* species showed that flavonoids such as kaempferol, quercetin, isorhamnetin and their glycoside derivatives were present (Jay et al., 1978; Reynaud et al., 1982; Strittmatter et al., 1992;

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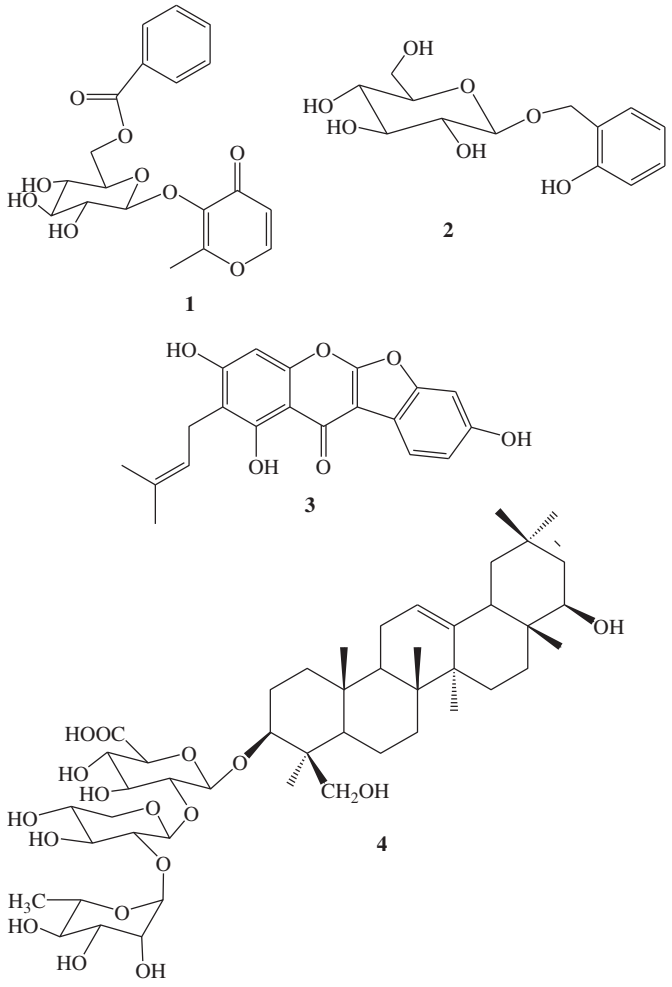
El Mousallami et al., 2002; El Youssef et al., 2008). Proanthocyanidins (Foo et al., 1997), phytoalexin isoflavans (Bonde et al., 1973; Ingham, 1977; Ingham and Dewick, 1979, 1980) and isoflavonoids (Yang et al., 1989; Abdel-Kader et al., 2006) have also been isolated from this genus. Chemical studies on related genera in the Fabaceae, *Astragalus*, *Medicago* and *Lupinus* genera have identified oleanan- and cycloartan-type triterpenoid glycosides (Jurzysta et al., 1992; Simonet et al., 1999; Avunduk et al., 2008). To date only a crude saponin was isolated from the *Lotus* genus (Walter, 1961).

3. Present study

In a continuation of our work on the chemical constituents of Algerian Saharan plants, the aerial parts of *L. pusillus* Medik. were investigated. We reported in the present study the isolation of maltol glucoside (**1**), phenolic glucoside (**2**), one isoflavonoid (**3**) and one saponin (**4**), together with β-sitosterol, β-sitosterol-3-*O*-β-D-glucopyranoside, β-sitosterol-3-*O*-6′-palmitoyl-β-D-glucopyranoside, oleanolic acid and taraxasterol. Structures of the isolated compounds have been determined on the basis of 1D and 2D homo- and heteronuclear NMR, ESI mass spectrometry and comparison to the respective literature data.



3.1. Extraction and isolation of constituents

The dried aerial parts of *L. pusillus* Medik. (700 g) were extracted twice with 5 L of petroleum ether at room temperature during 4 days. The residue was extracted successively with 2 × 5 L ethyl acetate and methanol at room temperature during 4 days. The ethyl acetate extract (7 g) was submitted to vacuum liquid chromatography (VLC) on silica gel using a gradient of petroleum ether–ethyl acetate 95:5, 90:10, 80:20, 70:30, 60:40, 50:50, 60:40, 70:30, 80:20, 90:10, ethyl acetate, ethyl acetate–methanol 99:1, 97:3, 95:5, 90:10, 80:20, 70:30, 60:40 and methanol, to afford 9 fractions. Fraction 6 (90 mg) was subjected to

a silica gel column chromatography using a gradient of petroleum ether–ethyl acetate (99:1, 97:3, 95:5, 90:10, 80:20, 50:50, 30:70) and ethyl acetate, to give two sub-fractions. Sub-fraction 1 (50 mg) showing two major components was submitted to purification by silica gel column eluted with a gradient petroleum ether–ethyl acetate (100% petroleum ether, then increment of 5%) afforded taraxasterol (12 mg) and β-sitosterol (20 mg). The sub-fraction 2 (90 mg) presented also two major products, and was further purified on a silica gel column using mixtures of petroleum ether and ethyl acetate (99:1, 98:2, 95:5, 90:10, 80:20, 70:30) allowed isolation of the pure separated compounds **3** (9 mg) and oleanolic acid (45 mg). Fraction 7 (60 mg) showing two major components was submitted to purification by silica gel column. Elution performed first with chloroform, then by a gradient of chloroform-methanol (99:1, 97:3, 95:5, 90:10 and 80:20) afforded β-sitosterol-3-*O*-6′-palmitoyl-β-D-glucopyranoside (7 mg) and β-sitosterol-3-*O*-β-D-glucopyranoside (16 mg).

The methanol extract was evaporated to dryness to give a brown gum (25 g) of which 7 g were submitted to vacuum liquid chromatography (VLC) on silica gel RP-18 using H_2O-methanol 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 and pure methanol, furnishing 7 fractions. Fraction 5 (150 mg) was applied to silica gel chromatography column eluting with chloroform–methanol (95:5, 90:10, 80:20, 70:30, 60:40) led to the isolation of compounds **1** (13 mg) and **2** (21 mg). Fraction 3 (400 mg) showing a major component was submitted to purification by column silica gel eluted with a gradient $CHCl_3$–MeOH–H_2O (90:10:0, 80:20:0, 70:30:0, 70:30:1, 70:30:3, 70:30:5 and 60:40:3) provided the pure compound **4** (15 mg).

3.2. Identification of constituents

Isolated compounds were identified by UV (Shimadzu UV-3101 spectrophotometer), IR (KBr, Shimadzu model IR-470 spectrometer), positive and negative ESI-MS (ion trap Bruker Esquire), 1D and 2D NMR analysis (COSY, HSQC, HMBC, Bruker Avance spectrometer, ^{1}H 500 MHz, ^{13}C 125 MHz) and by comparison with literature data. They were identified as maltol 3-*O*-[6-*O*-benzoyl]-β-D-glucopyranoside (**1**) (Nakano et al., 2011), 2-hydroxybenzyl-β-D-glucopyranoside (**2**) (Kitajima et al., 1998), Lupinalbin B (**3**) (Yang et al., 1989), astragaloside VIII (**4**) (Kitagawa et al., 1983), β-sitosterol (Nes et al., 1992), β-sitosterol-3-*O*-β-D-glucopyranoside (Voutquenne et al., 1999), β-sitosterol-3-*O*-6′-palmitoyl-β-D-glucopyranoside (Yili and Yuting, 1992), oleanolic acid (Voutquenne et al., 2003) and taraxasterol (Reynolds et al., 1986).

4. Chemotaxonomic significance

Molecular phylogenetic analyses show that tribe *Loteae*, to which the genus *Lotus* belongs, is monophyletic (Allan and Porter, 2000; Allan et al., 2003). *Lotus*, a large cosmopolitan genus (120–130 species), grows in two major centers of diversity, the mediterranean region (comprising areas of Europe, Africa and western Asia) and western North America termed Old and New World, respectively (Allan et al., 2004). However, recent studies based on morphological and nrITS data (Allan and Porter, 2000; Arambari et al., 2005) indicate that the New World species are not closely related to the Old World *Lotus* (Degtjareva et al., 2006). The genus *Lotus* (tribe *Loteae*) is native of the Old World. Its complex taxonomy has been subjected to several changes, particularly at the section level. The recent change in sectional classification of the Old World *Lotus* subdivides this genus into 14 sections (Degtjareva et al., 2006). *L. pusillus* Medik. (*L. halophilus* Boiss. & Spruner) belongs to the section *Loteae* which includes *Lotus cytisoides* L., *Lotus longisiliquosus* R. Roem., *Lotus ornithopodioides* L., *Lotus peregrinus* L., *Lotus polyphyllus* Clarke and *Lotus weilleri* Maire (Degtjareva et al., 2006), and the "Zygocalyx" clade (=clade F + G) (including sect. *Loteae* and *Lotus simonae* Maire, Weiller & Wilczek of sect. *Ononidium*). Species of this clade present generally a monosymmetric calyx (Degtjareva et al., 2006). The literature shows that isoflavonoids comprising prenylated isoflavonoids, and their glycoside derivatives are characteristic constituents of genera of the taxonomy-advanced subfamily Papilionoideae such as *Lotus*, *Euchresta*, *Piscidia*, *Lupinus* and *Andira* (Cagnin and Gottlieb, 1978; Tahara et al., 1985, 1993; Yang et al., 1989; Lo et al., 2002; Garcez et al., 2010). In this study, we have isolated one prenylated isoflavonoid named Lupinalbin B (3) which was previously obtained from *Lupinus albus* L. (Tahara et al., 1985) and *Lotus creticus* L. (sect. *Pedrosia*) (Yang et al., 1989). This result is in agreement with the chemical composition reported for the subfamily Papilionoideae. Soyasapogenol B glycosides are widely represented in species that belong to genera of this subfamily (as *Trifolium*, *Astragalus*, *Lupinus*, *Melilotus* and *Medicago*) and could be a chemotaxonomic character of this subfamily to which also the genus *Lotus* belongs (Kalač et al., 1996; Simonet et al., 1999; Hirakawa et al., 2000; Oleszek and Stochmal, 2002; Woldemichael and Wink, 2002; Avunduk et al., 2008). *L. pusillus* also contains the soyasapogenol B glycoside called astragaloside VIII (**4**). However, previous reports on the constituents of species of *Lotus* did not mention the presence of saponins, except in the case of *Lotus corniculatus* L. (sect. *Lotus*) where a small quantity of crude saponin was isolated, which upon hydrolysis yielded soyasapogenol B (Walter, 1961). To our knowledge this is the first time that saponin has been characterized in *Lotus*. Astragaloside VIII (**4**), already found in several genera of the subfamily Papilionoideae, seems to be a good chemotaxonomic character of this subfamily (Oleszek and Stochmal, 2002). Previous phytochemical studies showed that maltol glycosides are often present in Caryophyllaceae plants such as *Dianthus superbus* var. *longicalycinus* (Maxim.) F.N. Williams (Shimizu et al., 1982), *Tunica prolifera* (L.) Scop. (Plouvier et al., 1993) and *Silene vulgaris* (Moench) Garcke (Borris et al., 1985). They have been also found in the Fabaceae plants, maltol 3-*O*-[6-*O*-(3-hydroxy-3-methyl-glutaroyl)]-β-D-glucopyranoside from hairy root cultures of *Glycyrrhiza glabra* L. (Li et al., 2000) and 3-hydroxy-2-methyl-4H-pyran-4-one 3-*O*-[4′-*O*-*p*-coumaroyl-6′-*O*-(3-hydroxy-3-methyl-glutaroyl)]-β-D-glucopyranoside from the leaves of *Styphnolobium japonicum* (L.) Schott (Kite et al., 2007). Maltol 3-*O*-[6-*O*-benzoyl]-β-D-glucopyranoside (**1**), identified very recently from *Dianthus japonicus* Thunb. (Caryophyllaceae) (Nakano et al., 2011) is described for the first time in the *Lotus* genus. Previous studies showed the occurrence of phenolic glycosides in several species of the

Fabaceae family such as *Onobrychis viciifolia* Scop. (Lu et al., 2000), *Eriosema tuberosum* (Ma et al., 1999) and *Amburana cearensis* (Allemão) A.C. Sm. (Bravo et al., 1999). The phenolic glycoside 2-Hydroxybenzyl-β-D-glucopyranoside (**2**) previously isolated from *Salix acmophylla* Boiss. (Salicaceae) (Iqbal et al., 2004) is also described to our knowledge for the first time in the *Lotus* genus.

Our work, which includes isoflavonoids, saponins, maltol derivatives, provides data for the chemotaxomical studies in the *Loteae* section. Up to now only proanthocyanidin content was used as a tool to differentiate among species of *Lotus* (Escaray et al., 2012).

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